

## EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	10/659326	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/01/05 10:05
L2	45	CCCCAA	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/01/05 10:11
L3	3	linear chromosome splitting vector	US-PGPUB; USPAT; EPO; JPO; DERWENT	WITH	ON	2007/01/05 10:10
L4	6	Harashima Satoshi	US-PGPUB; USPAT; EPO; JPO; DERWENT	NEAR	ON	2007/01/05 10:10
L5	32	I2 and chromosome	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/01/05 10:16
L6	29	I2 and yeast	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/01/05 10:16

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(FILE 'HOME' ENTERED AT 10:39:36 ON 05 JAN 2007)

FILE 'MEDLINE, SCISEARCH, CAPLUS, BIOSIS' ENTERED AT 10:44:15 ON 05 JAN 2007

L1 E HARASHIMA SANTOSHI/AU  
198 S E4  
E SUGIYAMA M/AU  
E SUGIYAMA MINETAKA/AU  
L2 34 S E3  
L3 24 S L1 AND L2  
L4 11 DUP REM L3 (13 DUPLICATES REMOVED)  
L5 33 S CCCCAA  
L6 4 S L5 AND YEAST  
L7 2 DUP REM L6 (2 DUPLICATES REMOVED)  
L8 18 S SPLIT? CHROMOSOME (L) YEAST  
L9 6 DUP REM L8 (12 DUPLICATES REMOVED)  
L10 6 SORT L9 PY

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L4 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN  
TI Linear chromosome splitting vector comprising target sequence, marker gene or centromere sequence and (C4A2)<sub>n</sub> sequence for modifying yeast chromosomes  
SO Eur. Pat. Appl., 49 pp.  
CODEN: EPXXDW  
IN Harashima, Satoshi; Sugiyama, Minetaka; Kaneko, Yoshinobu  
AB The present invention provides a method of modifying yeast chromosomes using linear chromosome splitting vectors. The method of the invention includes preparing a first linear chromosome splitting vector comprising a first target sequence, a marker gene sequence, and a first (C4A2)<sub>n</sub> sequence; preparing a second linear chromosome splitting vector comprising a second target sequence, a centromere sequence of a chromosome, and a second (C4A2)<sub>n</sub> sequence; and introducing the chromosome splitting vectors into a cell, wherein n is independently an integer of 1 to 30, preferably 4-15, more preferably 6-10. The invention relates to PCR and primers for construction of chromosome splitting vectors. Yeast chromosome could be split sequentially into five chromosomes.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1422295	A1	20040526	EP 2003-256936	20031103
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
JP 2004166654	A	20040617	JP 2002-339259	20021122
US 2004224415	A1	20041111	US 2003-659326	20030911

L4 ANSWER 6 OF 11 MEDLINE on STN DUPLICATE 5  
TI A versatile and general splitting technology for generating targeted YAC subclones.  
SO Applied microbiology and biotechnology, (2005 Nov) Vol. 69, No. 1, pp. 65-70. Electronic Publication: 2005-10-20.  
Journal code: 8406612. ISSN: 0175-7598.  
AU Kim Yeonhee; Sugiyama Minetaka; Yamagishi Kazuo; Kaneko Yoshinobu; Fukui Kiichi; Kobayashi Akio; Harashima Satoshi  
AB Yeast artificial chromosomes (YAC) splitting technology was developed as a means to subclone any desired region of eukaryotic chromosomes from one YAC into new YACs. In the present study, the conventional YAC splitting technology was improved by incorporating PCR-mediated chromosome splitting technique and by adding autonomously replicating sequence (ARS) to the system. To demonstrate the performance of the improved method, a 60-kb region from within a 590-kb YAC (clone CIC9e2 from Arabidopsis thaliana

chromosome 5) that could not be subcloned using the original method was split to convert into a replicating YAC. Two template plasmids, pSK-KCA and pSKCLY, were used to generate two splitting fragments by PCR. Two splitting fragments consisted of telomeric (C(4)A(2))(6) repeats, 400-bp target region, CEN4, H4ARS and Km(r) (selective marker for plant transformants), or CgLEU2. These splitting fragments were introduced into *Saccharomyces cerevisiae* harboring the 100-kb split YAC generated by splitting of the 590-kb YAC and containing the 60-kb region. Among 12 Leu(+) transformants, four exhibited the expected karyotype in which two newly split 40- and 60-kb chromosomes were generated. These results demonstrate that the improved method can convert a targeted region of a eukaryotic chromosome within a YAC into a replicating YAC.

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